15:42:44 OCA PAD AMENDMENT - PROJECT HEADER INFORMATION 01/13/94

Active

Project #: G-33-625 Cost share #: Rev #: 4
Center # : 10/24-6-R6834-3A0 Center shr #: OCA file #:
Work type : RES
Contract#: 5 R01 DA06305-03 Mod #: CARRY FORWARD Document : GRANT
Prime #: Contract entity: GTRC

Subprojects ? : N CFDA:
Main project #: PE #: N/A

Project unit: CHEMISTRY Unit code: 02.010.136
Project director(s):
ZALKOW L H CHEMISTRY (404)894-4045

Sponsor/division names: DHHS/PHS/ADAMHA / ALCOHOL, DRUG ABUSE & MENTAL
Sponsor/division codes: 108 / 004

Award period: 911201 to 930831 (performance) 930831 (reports)

Sponsor amount	New this change	Total to date
Contract value	(5,639.64)	223,044.36
Funded	(5,639.64)	223,044.36
Cost sharing amount		0.00

Does subcontracting plan apply ?: N

Title: IRREVERSIBLE ANTAGONISTS OF COCAINE AND OTHER STIMULANTS

PROJECT ADMINISTRATION DATA

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Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): N
Defense priority rating : N/A NIH supplemental sheet
Equipment title vests with: Sponsor GIT X

Administrative comments -
CARRY FORWARD OF $5,639.64 TO -04 YEAR OF CONTINUING GRANT. SEE G-33-E50.

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 02/21/94

Project No. G-33-625 Center No. 10/24-6-R6834-3A0

Project Director ZALKOW L H School/Lab CHEMISTRY

Sponsor DHHS/PHS/ADAMHA/ALCOHOL, DRUG ABUSE & MENTAL

Contract/Grant No. 5 R01 DA06305-03 Contract Entity GTRC

Prime Contract No.

Title IRREVERSIBLE ANTAGONISTS OF COCAINE AND OTHER STIMULANTS

Effective Completion Date 930831 (Performance) 930831 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	N	
Final Report of Inventions and/or Subcontracts	N	
Government Property Inventory & Related Certificate	N	
Classified Material Certificate	N	
Release and Assignment	N	
Other	N	

CommentsCONTINUED BY G-33-E50.

Subproject Under Main Project No.

Continues Project No. G-33-608

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
Other	N
	N

B. METHODOLOGICAL CONSIDERATIONS

The techniques described in the original grant for the biochemical and behavioral characterization of the synthesized compounds will be retained, except that [^{3}H]CFT instead of [^{3}H]methylphenidate binding will be used to characterize the activity of the potential cocaine antagonists at the stimulant receptor. Due to its higher affinity and slower dissociation rate compared to cocaine[43], [^{3}H]CFT has become a popular radioligand for the characterization of the cocaine receptor on the dopamine transporter. [^{3}H]methylphenidate binds to a closely related stimulant binding site, but its relatively low affinity (K_D ~100 nM) and rapid dissociation rate[6] have led to its discontinuation as a commercially available radioligand. We feel that use of [^{3}H]CFT in these studies will better facilitate comparison of the data generated here with that from other laboratories. Because the two probes may bind to slightly different sites on the transporter (see discussion of the effect of sulfhydryl reagents on stimulant binding, above), however, determination of the inhibition of [^{3}H]methylphenidate binding by compounds found to antagonize the behavioral effects of cocaine in these studies may eventually be desirable.

3. PROGRESS REPORT/PRELIMINARY STUDIES

A. LIST OF ALL PERSONNEL.

Name	Title	Birthday	SS No.	Dates of service	% time
Deutsch,HM	Senior Research Sci.	4-23-40	119-32-5569	12-90 to present	50-75
Zalkow,LH	Regents' Professor	11-27-29	255-36-9515	12-90 to present	8
Kowalik, EG	Research Scientist	8-6-51	450-55-0969	12-90 to 11-91	83
Hernandez, M	Postdoctoral Fellow	5-1-55	382-62-3549	12-90 to 11-91	21
Lee, H	Graduate Student	12-25-65	172-54-4934	12-90 to 11-91	79
Sepcic, K	Graduate Student	12-6-67	247-49-9411	11-91 to 6-92	100
Huang, F-Y	Postdoctoral Fellow	2-26-56	004-80-2054	11-91 to 6-92	80
Bharathi, S	Postdoctoral Fellow	5-10-54	509-96-3189	11-91 to 3-92	100
Li, L	Technician	11-13-56	368-11-5346	5-92 to 6-92	50
Schweri, M	Associate Professor	8-29-46	404-60-6486	12-90 to 6-92	40
Cassidy, M	Research Technician	10-20-42	436-68-3042	12-90 to 4-92	100

B. PREVIOUS SPECIFIC AIMS.

The purpose of the previously proposed work was to synthesize compounds which would antagonize the reinforcing and psychosis-inducing properties of cocaine and other stimulants by binding irreversibly to sites on or related to the dopamine transporter. This approach was based on previous findings that Metaphit, a compound which *in vitro* irreversibly inhibits binding to recognition sites for the stimulant drugs cocaine and methylphenidate and irreversibly blocks dopamine transport, prevents the increased locomotor behavior normally seen following the administration of cocaine and other compounds belonging to the methylphenidate class of stimulant drugs.

Confirmation of this hypothesis would be sought by testing the drug Fourphit, which is known to bind irreversibly to the stimulant binding site, but not to the phencyclidine binding site. The compounds selected for modification were mazindol, the "GBR" series of aryl 1,4-dialk(en)ylpiperazines, methylphenidate, and dopamine itself. The reactive groups to be introduced into their structures were isothiocyanate, bromoacetamide, maleimide, and fluorosulfonyl.

The ability of the newly synthesized compounds to irreversibly block the actions of cocaine and other stimulants at the dopamine transporter would be tested by examining their behavior in the [^{3}H]methylphenidate radioreceptor assay. The compounds would also be tested *in vitro* for their ability to irreversibly block [^{3}H]dopamine uptake.

Those compounds which were found to irreversibly inhibit stimulant binding and dopamine uptake with some degree of selectivity would then be tested *in vivo* for their behavioral effects. Activity of the compounds would be examined in three separate behavioral paradigms. Compounds which showed behavioral activity would then be tested *ex vivo* for their ability to block [^{3}H]methylphenidate binding and [^{3}H]dopamine uptake.

C. SUMMARY OF RESULTS LEADING TO PROGRESS TOWARDS THE SPECIFIC AIMS.

1. Fourphit

a. Biochemical studies.

i. *In vitro*. Studies examining the effect of Fourphit on [^{3}H]methylphenidate binding[6] showed that this isothiocyanate derivative of phencyclidine inhibited binding to the stimulant recognition site with an IC_{50} of 7.1 μM. Washout studies (conducted under conditions where reversibly bound phencyclidine was completely removed) demonstrated that inactivation of the stimulant recognition site by Fourphit was irreversible, while inactivation of the phencyclidine receptor by the compound was reversible. Partial protection of the stimulant recognition site from inactivation by Fourphit was afforded by pretreatment of striatal tissue with saturating amounts of unlabeled methylphenidate, suggesting that the acylating compound may bind covalently to the receptor directly at the site recognized by methylphenidate, rather than allosterically. Studies examining the effects of Fourphit on the dissociation rate of [^{3}H]methylphenidate also supported the interpretation that the inhibition caused by Fourphit was not due to an allosteric interaction of the compound with the stimulant recognition site.

ii. *Ex vivo*. Measurement of [^{3}H]methylphenidate binding in striatal tissue dissected from the rats utilized in the behavioral studies described below revealed no consistent changes supportive of altered dopamine transport function.

b. Behavioral studies. As predicted in the original proposal, Fourphit attenuated the hyperactivity induced by challenge with a low dose of cocaine[7]. Intravenous injection of Fourphit (20 mg/kg) had little effect on the activity levels of rats. The effect of Fourphit on stimulant-induced locomotion appeared to vary with the respective stimulants. Rats pretreated with Fourphit prior to challenge with methylphenidate (Ritalin), which belongs to the same class of stimulant agents as cocaine, however, showed no change (or possibly even a slight increase) in activity, compared to vehicle-pretreated control animals injected with methylphenidate. Pretreatment with Fourphit significantly increased locomotor activity observed following low doses of d-amphetamine (0.8 and 1.25 mg/kg, respectively), while it was without effect on the activation induced by 1.5 mg/kg of methamphetamine, which belongs to the same class of stimulant drugs as amphetamine.

In conclusion, the results with fourphit generally support our hypothesis that drugs that bind irreversibly to the stimulant binding sites can act as antagonists to cocaine.

2. GBR Series

a. Synthesis (Scheme 1)[9]. The reaction of piperazine with cinnamyl bromide gave both the desired compound **1** as well as the dialkylated material **4** which can be separated by distillation. Amine **1** was then alkylated with chloroether **2**, which was made by reduction of the corresponding benzophenone followed by etherification with chloroethanol. Selective reduction of the nitro group of **3a** or **3b** yielded the amines **3c** or **3d** which served as the basic starting materials for the synthesis of the potential irreversible compounds. Isothiocyanates **3e** and **3f** and maleimides **3g** and **3h** were prepared by standard methods. Using a similar method, but starting with fluoreneone, compound **5** was synthesized.

After the synthesis of the 3'-amino derivative **6b** had been initiated (by the scheme shown), we learned that colleagues at NIH (K. Rice and B. deCosta) had already made this compound. Using material supplied by them, the synthesis of **6c** and **6d** was completed.

b. Pharmacology.

i. Binding studies

a. *In vitro* studies. The parent compound (GBR-12783) and synthetic compounds **3-6** were tested in the [^{3}H]methylphenidate binding assay (Table 1)[9]. The inhibition was marked by an apparent Hill coefficient greater than one in all cases. Washout studies using a sodium-free solution for removing reversibly-bound compounds showed that compounds **3e** and **3f** bound irreversibly to the stimulant recognition site, whereas **3g** showed partial irreversible inhibition and all of the other tested compounds were reversible. The time course for the reaction of **3f** with the [^{3}H]methylphenidate binding site was found to be extremely rapid, even at 0 °C. Scatchard analysis of the effect of **3f** on [^{3}H]methylphenidate binding suggest that the compound leads to a reduction in B_{max}. Protection of the site from irreversible inhibition by **3f** using saturating amounts of GBR-12783 was attempted. Complete protection using this technique was not observed.

TABLE 1.

Inhibition of [^{3}H]methylphenidate binding by GBR-12783 and its derivatives

Compound	IC_{50}(nM)	Hill Coefficient	Compound	IC_{50}(nM)	Hill Coefficient
GBR-12783	12.0 ± 1.2	3.03 ± 0.05	**3f**	493 ± 94	2.30 ± 0.13
3a	11.9 ± 1.2	1.55 ± 0.12	**3g**	970 ± 46	1.32 ± 0.18
3b	39.3 ± 4.7	1.56 ± 0.13	**3h**	1700 ± 412	1.90 ± 0.29
3c	15.8 ± 1.3	1.89 ± 0.06	**4**	2000	ND
3d	15.4 ± 0.9	1.89 ± 0.05	**5**	1700	ND
3e	283 ± 18	1.51 ± 0.08	**6b**	48	ND

b. *Ex vivo* studies. [^{3}H]methylphenidate binding studies were conducted on the striatal tissue of the rats used in the behavioral studies summarized below. The rats were sacrificed shortly after the termination of each behavioral experiment, the tissue was removed and frozen until the series was completed, at which time all the samples were analyzed using the same radioreceptor assay. No reduction in [^{3}H]methylphenidate binding was observed as a function of treatment. However, further statistical analysis of one experimental series suggested a negative correlation between binding and activity of the individual rats treated with **3e**, while rats treated with vehicle in the same experiment showed a possible positive correlation between binding and activity.

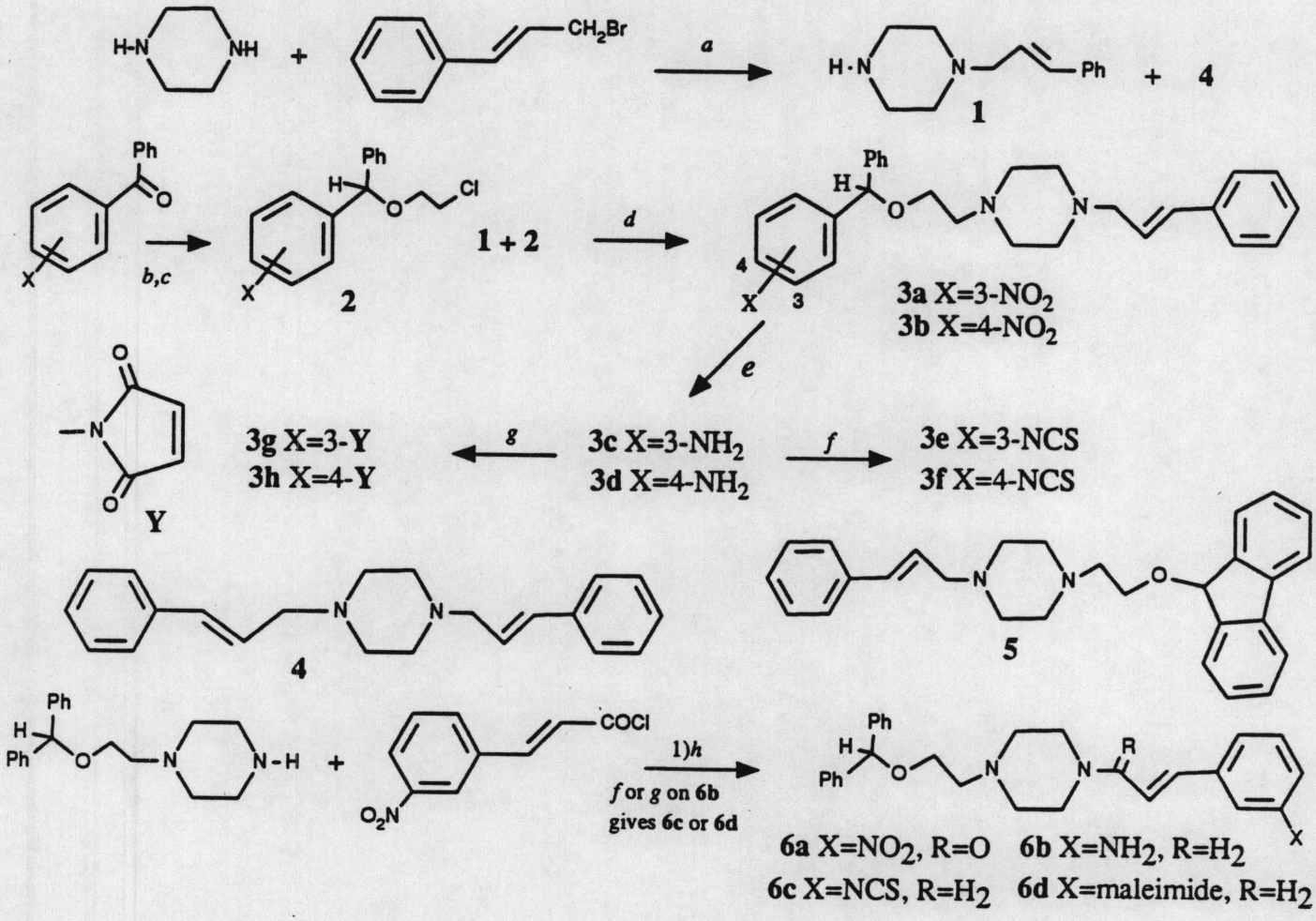
ii. [^{3}H]Dopamine transport studies. The potency of test compounds in inhibiting [^{3}H]dopamine transport into striatal synaptosomes was assessed. IC_{50}'s shown below are derived from inhibition curves in which inhibition at five to seven different concentrations of test compound was determined. The time course for inactivation of [^{3}H]dopamine transport by **3f** has been examined and indicates that maximum inhibition occurs within 5-10 min at 0 °C.

Compound	IC_{50}(nM)
GBR-12783	5.5
3e	108
3f	92
3h	380

iii. Behavioral studies. The effects of **3e** and **3f** on the hyperactivity caused by low dose cocaine and amphetamine were examined and these results are summarized in Table 2 (Fourphit and mazindol derivative **11d** are included in this table for convenience). Despite solubility problems with some of these compounds which limited the amount of drug which could be delivered by certain

routes, some interesting results were obtained. The test compounds were delivered by intravenous and intraperitoneal injection, as well as microinjection directly into the nucleus accumbens. In some experimental paradigms, **3e** and **3f** potentiated the effects of low dose cocaine; no conditions were found in which it blocked cocaine's behavioral effects. Compound **3e** was ineffective in preventing the stimulation caused by *d*-amphetamine.

Scheme 1



a. K_2CO_3, EtOH; *b*. $NaBH_4$, EtOH; *c*. $HOCH_2CH_2Cl$, H_2SO_4; *d*. K_2CO_3, KI, CH_3CO-*i*Bu, heat; *e*. $NaBH_4 \cdot 3S$, THF, Δ; *f*. $CSCl_2/HCCl_3$, $NaHCO_3/H_2O$; *g*. 1) maleic anhydride, THF 2) NaOAc, Ac_2O; *h*. pyridine as solvent gives **6a**; condition (*e*), followed by $LiAlH_4$ gives **6b**.

In summary, there was no evidence that irreversible binding GBR-like compounds could act as antagonists to cocaine. However, the 3'-isothiocyanate and maleimide (**6c** and **6d**) have not yet been tested. Isothiocyanate groups at the *meta*- or *para*- position led to a 20- and 40-fold reduction in binding potency, respectively whereas maleimido groups at these positions led to 80- and 140-fold reductions in binding potency, respectively. Both the 3- and 4-amino and nitro-substituted intermediates showed only small decreases in binding potency, compared to the parent compound. Since the nitro group is strongly electron withdrawing and the amino group is strongly electron releasing, this indicated that electronic factors are only of minimal importance. The marked decrease in binding potency for the bulkier maleimide and isothiocyanate groups indicate that steric factors appear to dominate. This is also confirmed by the low binding potency of the restricted rotation analog **5**. In fact, since the IC_{50} value for **3h** is appoximately the same as that for both **4** and **5** it appears that the

4-maleimide group essentially blocks the binding at this end of the molecule. Since these are racemic compounds this implies that both phenyl rings comprising the geminal diphenyl portion of the molecule (see structure **3**) are involved in the binding interaction. If this were not true, then we would expect one of the isomers (of the *d,l* pair) to have a binding potency similar to the unsubstituted compound (GBR-12783) and therefore the racemic mixture would have no more than a two-fold reduction in binding potency. The washout conditions used to evaluate the irreversibility of the inhibition of [^{3}H]methylphenidate binding by these compounds led to the complete removal of reversibly bound inhibitors only when potassium chloride was substituted for sodium chloride in the wash solution.

Table 2. Summary of the effects of test compounds on stimulant-induced hyperactivity.

TEST COMPOUND	DOSE (mg/Kg)	ROUTE	CHALLENGED WITH:	AT(HRS)	SEX	RESULTS
FOURPHIT	20	IV	COCAINE	24	F	⇓
3e	2	IV	COCAINE	24	M	⇔
3e	2	IV	COCAINE	0.5	M	⇑
3e	7.6 nmols	intra-accumbens	COCAINE	24	M	⇔
3e	2	IV	D-AMPHET	24	M	⇔
3f	2	IV	COCAINE	24	M	⇔
3f	2	IV	COCAINE	0.5	M	⇔
3f	25	IP	COCAINE	24	M	⇑
3f(NBR RATS)	25	IP	COCAINE	24	M	⇔
11d	1.25	IV	COCAINE	0.25	M	⇔

Cocaine dose was 15 mg/kg, i.p., as the hydrochloride salt. All studies done in sprague-Dawley rats, except where noted. Rats were challenged with stimulant at the time shown in hours after injection with the test compound. Activity was compared to rats injected with vehicle instead of test compound, before challenge with the stimulant. Results show change in activity with respect to stimulant injected controls (⇓, decrease;⇔, no change;⇑, increase). Six to eight rats were in each treatment group.

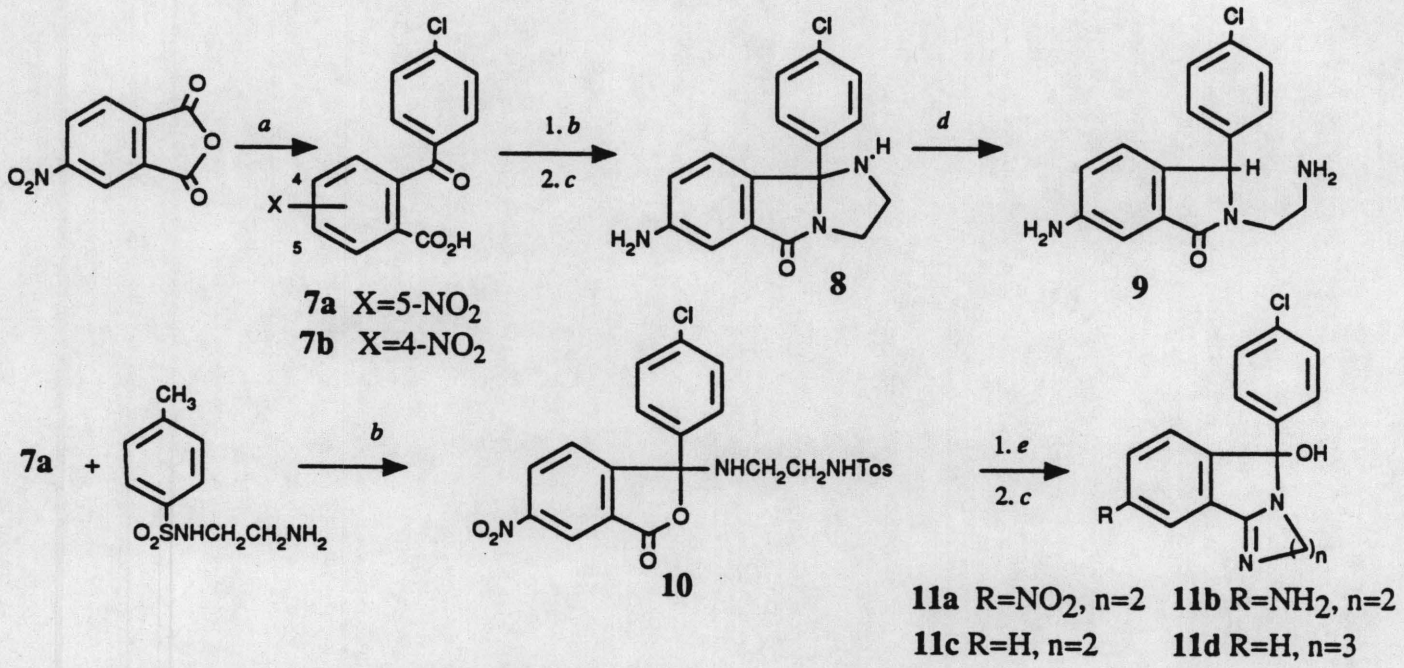
3. Mazindol Series

a. Synthesis (Scheme 2). The Friedel-Crafts acylation of chlorobenzene with 4-nitrophthalic anhydride gave a 4:1 ratio of the 5-nitro product (**7a**) to the 4-nitro product (**7b**), which could be separated by several methods. Condensation of **7a** with ethylene diamine followed by catalytic reduction gave **8**. Lithium aluminum hydride reduction did not give the expected product; instead a material formulated as **9** was produced which could not be converted to a mazindol. However, the synthesis of both mazindol (**11c**) and the ring expanded product **11d** was accomplished using this method.

A new methodology, based on work in the patent literature started with **7a** and the monotosylate of ethylenediamine. Compound **10** could be converted to nitromazindol (**11a**) in good yield as indicated below. Catalytic reduction of **11a** to **11b**, without reduction of the carbon-nitrogen

double bound, appeared to be successful although a pure product has not yet been isolated. We are currently attempting to purify **11b** and convert it to a potential irreversible binding compound.

Scheme 2



a. Chlorobenzene, $AlCl_3$; *b*. Ethylenediamine, toluene, heat (remove water); *c*. H_2/Pd *d*. $LiAlH_4/Et_2O$; *e*. Conc. H_2SO_4, 70 °C.

b. Pharmacology.

i. Binding studies.

Compound	IC_{50}-Nm
mazindol (**11c**)	16
11a	2300
11d	5

ii. Behavioral studies. Since the mazindol derivative **11d** (with an expanded imidazole ring) had been reported earlier to have high binding potency, but no behavioral effect (and thus a potential antagonist), its influence on the hyperactivity caused by low dose cocaine were examined. The results are summarized in the table 1, and show no effect on cocaine induced behavior.

In summary, the synthesis of irreversible mazindol derivatives is nearing completion at this time. Although the nitro compound **11a** shows greatly reduced binding potency, this may be due to electronic factors that would not be present in the amino compound. Interestingly, the ring expanded mazindol **11d** shows increased binding potency, but no behavioral effects.

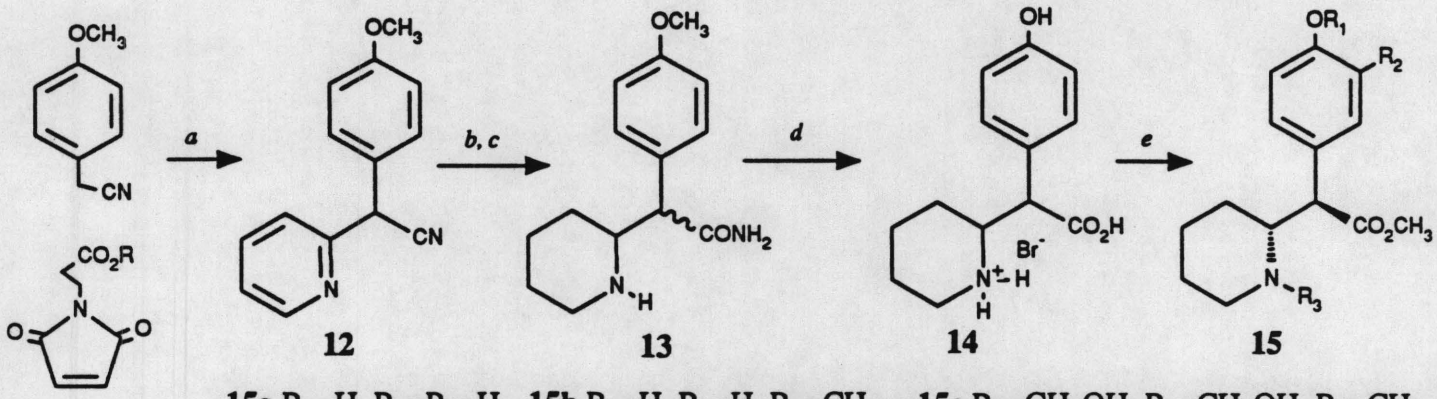
4. Methylphenidate Series

a. Synthesis (Scheme 3). The reaction of p-methoxyphenylacetonitrile with 2-bromopyridine according to a literature procedure gave a difficult to separate mixture from which the desired product (**12**) could be separated only by chromatography in very low yield. After modification of the conditions, satisfactory yields (isolated by crystallization) were obtained as shown below. Partial hydrolysis of **12** gave an amide which upon hydrogenation gave **13** as a mixture of *erythro* (predominately) and *threo* isomers. Deprotection and isomerization of mixture **13** was achieved in excellent yield using 48% HBr which yielded **14**, in which the predominant *threo* isomer could be separated by crystallization. Conversion of *threo* **14** to the methyl ester yielded

p-hydroxymethylphenidate (**15a**). In order to prevent problems with the synthesis of potential irreversibly binding compounds, the synthesis of N-methyl *p*-hydroxymethylphenidate was attempted. Reductive amination (with formaldehyde and sodium cyanoborohydride) gave a mixture of products in low yield which could not be separated (may have been an *erythro/threo* mixture). Clark-Eschweiler conditions gave a mixture from which the N-methyl compound (**15b**) and the *bis*-hydroxymethyl compound (**15c**) could be isolated.

The ethyl ester of maleimido ß-alanine ethyl ester (**16a**) has been synthesized and it has been hydrolyzed to the free acid **16b**. Current work involves coupling **16b** to the protected compound **15d**, which after deprotection would yield the potential irreversibly binding compound **15e**.

Scheme 3



12 **13** **14** **15**

15a R_1=H, R_2=R_3=H **15b** R_1=H, R_2=H, R_3=CH_3 **15c** R_1=CH_2OH, R_2=CH_2OH, R_3=CH_3

16a R=Et

16b R=H

15d R_1=H, R_2=H, R_3=t-BOC **15e** R_1=maleimido-β-alanyl, R_2=R_3=H

a. t-BuOK, toluene, 2-bromopyridine; *b*. Pt/HOAc/H_2; *c*. conc. H_2SO_4; *d*. 48% HBr; *e*. MeOH/HCl

b. Pharmacology

a. Binding studies

Compound	IC_{50}-nM
Methylphenidate	95
15a	115
15b	1080
15c	443

In summary, it has been shown that some substituents in the *para* or *meta* position of the benzene ring of methylphenidate do not significantly affect binding affinity, whereas an N-methyl substituent leads to an appproximate 10-fold lowering of affinity.

5. Dopamine series

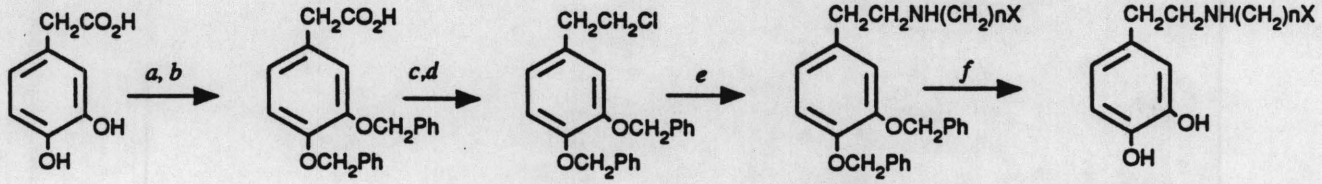
a. Synthesis (Scheme 4). The benzylation and subsequent hydrolysis of dihydroxyphenylacetic acid yielded the protected acid in relatively low yield. Reduction of the acid and formation of the chloride **17** proceeded smoothly as did the subsequent alkylation to form diamines **18a-c** and after deprotection catechols **19a-c**. Bromoacetamide derivative **18d** was readily synthesized and purified; although **19d** has been made, a pure product has yet to be isolated.

b. Pharmacology. Washout studies showed that the bromoacetamide **18d** irreversibly inhibits binding, while the unprotected precursor **19a** does not. Surprisingly, **18a** (which has no reactive group) also appeared to irreversibly inhibit binding, even under conditions[9] shown to effectively remove "pseudo-irreversible" inhibitors like GBR-12783. The potencies of these compounds against dopamine transport was comparable to their activities at the methylphenidate binding site.

Preliminary experiments suggest that **18d**, the only derivative tested thus far against dopamine transport, is an irreversible inhibitor.

	----------IC_{50}-μM----------	
Compound	Binding	[^{3}H]Dopamine Uptake
dopamine	3.8	0.15
18a	3.6	2.8
18b	1.6	ND
18c	1.5	ND
18d	13.0	3.1
19a	5.1	4.1

Scheme 4



17 **18a-d** **19a-d**

18a and **19a** n=2, X=NH_2 **18c** and **19c** n=4, X=NH_2
18b and **19b** n=3, X=NH_2 **18d** and **19d** n=2, X=$NHCOCH_2Br$

a. NaH/DMF, BnBr; b. KOH, EtOH/H_2O; c. B_2H_6/THF; d. $SOCl_2$; e. ex. $H_2N(CH_2)_nNH_2$/toluene; f. Pd/C/EtOH/H_2. Note: derivatives **18d** and **19d** were made from **18a** and **19a**, respectively, with bromoacetyl bromide.

In summary, the diamine derivatives **18a-18d** and **19a** bind with similar potency as dopamine. Interestingly, the *bis*-benzyl protected intermediates exhibited somewhat increased affinity, whereas the acylated derivative **18d** is somewhat less potent. Compound **18a** appears to be a psuedoirreversible inhibitor based on the inability to wash it out of tissue preparation under conditions that are effective for the GBR series. The effects of this psuedoirreversible compound on behavior will be tested shortly. At the present time it is not known if the bromoacetamide derivative **18d** truly binds irreversibly or is just difficult to washout.

6. General Pharmacology. The following information was gained:

a. Similar to other stimulant binding, [^{3}H]methylphenidate binding increases in phosphate buffer compared to Tris buffer.

b. The stimulant drug amfonelic acid is a competitive inhibitor of the high affinity [^{3}H]methylphenidate binding site.

c. The stimulant drug nomifensine has an IC_{50} of 38 nM at the [^{3}H]methylphenidate binding site. When the primary aromatic amine of nomifensine is converted to an isothiocyanate group the IC_{50} increased to 1360 nM; washout studies showed it was not binding irreversibly.

d. When examined over 20 h, [^{3}H]methylphenidate binding is severely reduced. Studies showed it was not due to deterioration of the radioligand, suggesting that either the receptor itself is degrading, or an inhibitory compound is being elaborated. This differs from studies with [^{3}H]GBR and [^{3}H]mazindol compounds, whose binding is relatively constant over 20 h.

D. CHANGES IN THE SPECIFIC AIMS FOR NEW PROPOSAL (UNDERLINED CHANGES).

The purpose of the proposed work is to synthesize and evaluate compounds which interact

with the stimulant binding site(s) on the dopamine transporter, with the aim of identifying compounds which are antagonists or partial agonists for the reinforcing and/or psychotogenic properties of cocaine.

1. Derivatives of LR5182, a compound with some structural similarities to cocaine, but higher selectivity and affinity for the dopamine transporter, will be synthesized.

2. Dual substrate. Several lines of evidence suggest that there may be more than one substrate and/or stimulant binding site per dopamine transport complex. Based on such a model, the effects of linking two dopamine molecules together and varying the distance of separation will be explored.

3. A GBR derivative with two reactive groups in the same molecule will be synthesized, with the aim that they might span two binding sites (or subunits).

4. New reversible GBR and Methylphenidate Analogs will be synthesized. The mutual inhibition of stimulant binding by GBR and methylphenidate with cocaine, suggests that derivatives of these stimulants may serve as antagonists or partial agonists for cocaine.

5. The synthesis of the 3-epoxide derivative of GBR-12783 wil test whether epoxides caan be used to target sulfhydryl groups.

6. The effect of the newly synthesized compounds on the dopamine transport complex will be determined by examining their ability to inhibit [^{3}H]-CFT, instead of [^{3}H]methylphenidate binding.

E. LIST ALL PUBLICATIONS SUBMITTED OR ACCEPTED THAT HAVE RESULTED FROM THIS PROJECT.

Deutsch, H.M., M.M. Schweri, C.T. Culbertson, and L.H. Zalkow, 1992, Synthesis and pharmacology of irreversible affinity labels as potential cocaine antagonists. 1. Aryl 1,4-dialkylpiperazines related to GBR-12783. Eur. J. Pharmacol., accepted pending revision.

Schweri, M.M., A. Thurkauf, M.V. Mattson and K.C. Rice, K.C., 1992, Fourphit: a selective probe for the methylphenidate binding site on the dopamine transporter, J. Pharmacol. Exp. Ther. 261, 936.

Abstracts/Presentations:

Schweri, M.M., Thurkauf, A., and Rice, K.C.: Fourphit binds irreversibly to the stimulant recognition site on the dopamine transporter. Abstracts, Twentieth Annual Meeting Soc. Neuroscience 16: 687, Abstr. #287.4, 1990.

Deutsch, H.M., Schweri, M.M., and Zalkow, L.H.: Cocaine antagonists. Synthesis of potential irreversible binding ligands for the methylphenidate binding site. Amer. Chem. Soc. Annual Meeting. 1991.

Schweri, M.M., Deutsch, H.M., Culbertson, C., and Zalkow, L.H.: A GBR-12783 derivative with potential as an irreversible cocaine antagonist. Abstracts, Seventy-fifth Annual Meeting, Fed. Amer. Soc. Expl. Biol. 5: A1590, Abstr. #7080, 1991.

Schweri, M.M., de Costa, B.R., and Rice, K.C.: Fourphit inhibits cocaine-induced hyperactivity in rats. Abstracts, Twenty-first Annual Meeting Soc. Neuroscience, 17: Abst. #348.3, 1991.

4. RESEARCH DESIGN AND METHODS

The proposed research will be conducted as follows: (1) Continuation of presently funded work on the synthesis of affinity labels and simultaneous initiation of work on new synthetic targets; (2) Simultaneous start of *in vitro* biochemical testing; (3) Behavioral testing when appropriate compounds are available; (4) *Ex vivo* biochemical testing. The procedures to be followed will be described under these headings in the text which follows.